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RAPID COMMUNICATION

Maternal Effects of the *short integument* Mutation on Embryo Development in *Arabidopsis*

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Maternal gene products deposited in an animal egg determine the polarity of embryonic axes and regulate embryonic cell–cell communication important for morphogenesis. Here we report the first maternal-effect embryo-defective mutation discovered in a plant. Recessive mutations in the *SHORT INTEGUMENT* (*SIN1*) gene in *Arabidopsis* were previously shown to influence ovule development and flowering time. Here we show that a *sin1* mutation has a pronounced maternal effect on zygotic embryo development. A homozygous *sin1* mutant embryo is normal when nursed by a *sin1*/+ heterozygous maternal sporophyte. Strikingly, a *sin1* or a *sin1*/+ embryo that is nursed by a *sin1* homozygous maternal sporophyte develops morphogenetic defects in the apical–basal and radial axes. The defects resemble those seen in some zygotic-effect embryonic pattern formation mutants. These results imply that in maternal cells the *SIN1* gene either codes for or controls the production of a diffusible morphogen necessary for proper zygotic embryogenesis. © 1996 Academic Press, Inc.

INTRODUCTION

Unlike animal eggs, the egg cell of flowering plants contains a relatively small amount of cytoplasm. Asymmetrically distributed maternal gene products within the large cytoplasm of an animal egg provide a polarized informational field for early pattern formation (for reviews see Nüsslein-Volhard *et al.*, 1987; St. Johnston and Nüsslein-Volhard, 1992; Chasan and Anderson, 1993). As yet there has been no evidence that maternal programming of embryonic morphogenesis occurs in plants. The earliest proposal for an alternative mechanism of morphogenesis, that of "self-organization" (Turing, 1952), described how an initially homogenous group of cells could become patterned by cell–cell communication without the need of a preformed polarized field. Artificial somatic embryogenesis in higher plants may indeed be an example of self-organization. Here, signaling within groups of somatic cells in culture enables the organization of morphologically normal embryos (Zimmerman, 1993; Vroemen *et al.*, 1996).

Since somatic embryogenesis is possible in plants, it is not clear whether an elaborate maternal mechanism needs to exist for specifying a zygotic embryonic pattern. The only known maternal-effect mutation in a plant, the *shrunken endosperm* mutations in barley, affects the endosperm and not the embryo (Felker *et al.*, 1985). A large number of embryo-lethal and pattern formation mutants were identified in *Arabidopsis* (Meinke, 1985; Mayer *et al.*, 1991), but technical limitations precluded the isolation of putative maternal-effect mutations. A maternal-effect embryo lethal mutation is one that has no effect on embryo viability when the homozygous mutant embryo develops within a heterozygous sporophyte. The lethal phenotype is expressed, however, when a heterozygous or a homozygous mutant embryo develops within a homozygous mutant sporophyte. Thus, a maternal-effect embryo-lethal mutation may be identified as a homozygous female sterile line in which embryo development is arrested even when the egg is fertilized by a sperm carrying the wild-type allele (West and Harada, 1993). A putative maternal-effect mutation in a plant is different from a female gametophytic mutation in its genetic behavior. For a female gametophytic mutation, the mutant allele from a heterozygous mother fails to be transmitted to the embryo. Here we show that *short integument* (*sin1*), a recessive female sterile mutation that is pleiotropic on flowering time (Robinson-Beers *et al.*, 1992; Lang *et al.*, 1994; Ray *et al.*, 1996), has a pronounced maternal effect that disrupts embryonic pattern formation. The maternal requirement of *SIN1* suggests that signaling from the maternal sporophytic tissues performs essential functions in normal plant embryogenesis.

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MATERIALS AND METHODS

The wild-type *Arabidopsis thaliana* strain WC1, the *sin1-2* mutant allele, conditions of seed germination, plant growth, propagation, and genetic and microscopic techniques have been described before (Lang *et al.*, 1994; Ray *et al.*, 1996). Confocal laser-scanning microscopy of embryos following staining with propidium iodide was performed essentially as described by Running *et al.* (1995). The *sin1-2* mutant plants show the same associated late-flowering phenotype as the original *sin1-1* allele (Ray *et al.*, 1996). Heterozygosity at *sin1* was first deduced by monitoring the polymorphism of a tightly linked (<0.5 c*M*) physical marker, nga59, in DNA isolated from seedlings and later confirmed by phenotypic and/or progeny analysis wherever possible. All strains were wild type for *ERECTA*, a mutation which is known to modify the *sin1* mutant phenotype (Lang *et al.*, 1994). The following crosses (see Table 1) were made: For cross 1a, flowers of the wild-type strain WC1 were pollinated with pollen from a homozygous *sin1-2*/*sin1-2* plant. For cross 1b, the pollen donor was a homozygous mutant for the stronger allele *sin1-1*. All the resulting progeny tested heterozygous for *sin1*. For crosses 2 and 6, flowers of *sin1-2*/*SIN1* plants were crossed to *sin1-2* pollen. Fifty percent of the resulting progeny tested heterozygous (cross 2), and the rest homozygous mutants (cross 6), at *sin1-2*. For cross 3, heterozygous flowers were crossed to wild-type pollen derived from WC1. Fifty percent of their progeny tested heterozygous (cross 3) and the rest was homozygous wild type. For crosses 4 and 5, *sin1-2*/*sin1-2* homozygous mutant flowers were crossed to either WC1 (cross 4) or *sin1-2* (cross 5) pollen. The surviving progeny of cross 4 tested heterozygous, and those from cross 5 tested homozygous mutants for *sin1-2*, respectively.

RESULTS AND DISCUSSION

Flowers homozygous for the hypomorphic allele *sin1-2* (Ray *et al.*, 1996) produce morphologically normal ovules at an approximate frequency of 30–50% per flower (Figs. 1A and 1B). These ovules mature into seeds upon pollination (Figs. 1C and 1D). To test for maternal effect, a series of crosses were made by emasculating unopened flower buds of plants with identified genotypes (Table 1), which were then pollinated with either *sin1-2* or wild-type pollen. The entire seed set derived from each cross was tested for germination on agar plates.

When the genotype of the recipient (maternal) flower is either homozygous or heterozygous for the wild-type *SIN1* allele, all crosses produce many seeds per flower. Nearly 100% of these seeds germinate to produce phenotypically normal seedlings that grow to maturity (Table 1, crosses 1–3 and 6). When the maternal sporophyte is a homozygous mutant, the frequency of seeds that germinate is low whether the embryo is homozygous or heterozygous for the *sin1-2* allele (crosses 4 and 5). Furthermore, most (>90%) seedlings that germinate on agar plates do not develop further. Thus, the wild-type *SIN1* allele when transmitted through the pollen is unable to rescue the deleterious effects on embryogenesis of a homozygous maternal *sin1-2* mutation.

In the seeds of flowering plants there are two independent zygotic tissues, the embryo and the endosperm, that are intimately connected and show mutual regulatory interactions (West and Harada, 1993; Hong *et al.*, 1996). The endosperm is genetically two-thirds maternal and one-third paternal. We show below that a wild-type allele of *SIN1* in the endosperm cannot rescue the maternal effect of *sin1-2*. In crosses 3 and 4 (Table 1), the genotypes of the embryo and the endosperm are identical. Cross 3 shows that two copies of the *sin1-2* allele in the endosperm have no deleterious effect on the embryo when the maternal sporophyte is heterozygous. Cross 4 shows that one wild-type allele in the endosperm is unable to rescue the effect of a homozygous mutant maternal sporophyte. Thus, a wild-type *SIN1* allele in the maternal sporophyte is necessary for normal embryogenesis irrespective of the genotype of the endosperm or the embryo. A possible role of the endosperm genotype in normal embryogenesis is eliminated by comparing the results of crosses 4, 5, and 6. Cross 5 demonstrates that a *sin1-2* homozygous mutant embryo growing within a homozygous mutant maternal sporophyte is no more affected than is a *sin1-2*/+ heterozygous embryo in a homozygous *sin1-2* sporophyte. Cross 6 shows that three copies of the mutant *sin1-2* allele in the endosperm have no adverse effect on a homozygous mutant embryo growing within a heterozygous maternal sporophyte. Cross 6 also demonstrates that a heterozygous maternal sporophyte fully supports normal development of the genotypically mutant endosperm and the embryo. Therefore, the presence of a wild-type *SIN1* gene in the maternal sporophyte is necessary and sufficient for normal embryogenesis. These experiments do not exclude the formal possibility that two or three copies of the functional *SIN1* gene in the endosperm may rescue the deleterious effects of a homozygous mutant maternal sporophyte on the embryo. This reservation does not detract from the main conclusion that expression of the *SIN1* gene in the mother is necessary for embryogenesis.

The egg cell of an angiosperm is usually polar in appearance, as is the zygote. Even the first-division plane of the zygote in *Arabidopsis* is asymmetric (Jürgens *et al.*, 1991), although it is somewhat variable among Angiosperms (West and Harada, 1993). Thus, the polarity of the embryonic axis in angiosperms may be specified early within a highly asymmetric embryo sac. It is also possible that the early specification processes leave a certain degree of plasticity that requires subsequent cell–cell communication to reinforce the final determination of the morphogenetic pattern. That this may be the case is indicated by the phenotypes of certain alleles of embryonic pattern formation mutations in which no strong correlation between early cell division pattern and the final morphology has been found (Berleth and Jürgens, 1993; Shevell *et al.*, 1994). It is an open question whether the diploid maternal cells that surround the embryo within an ovule play any role in directing embryonic pattern formation.

To determine whether the maternally expressed *SIN1* gene is important in pattern formation, seedlings derived from crosses with *sin1-2*/*sin1-2* flowers were analyzed for morphological defects. Of 49 seedlings so observed, 7 were

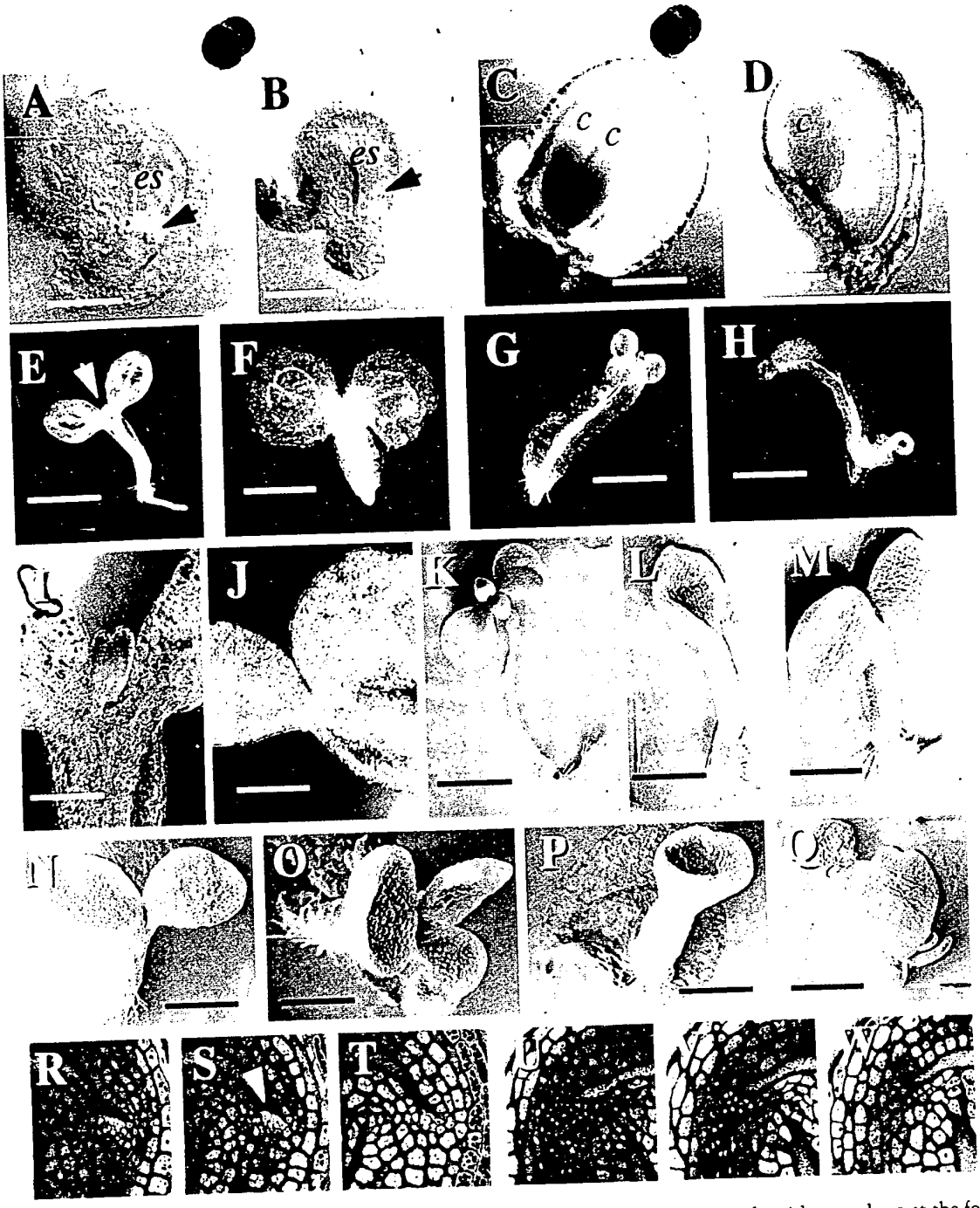

FIG. 1. Maternal effects of *sin1-2* mutation on embryonic pattern formation. (A) A wild-type ovule with an embryo at the four-cell stage (arrow). (B) A morphologically normal ovule with a four-cell stage embryo (arrow) in a *sin1-2/sin1-2* plant. Ovules of this morphology represent 30–50% of all ovules in each flower. (C) A wild-type seed. (D) A seed derived from a *sin1-2/sin1-2* self-cross. Note the single cotyledon of the mature embryo. (E–Q) Ten-day postgermination seedlings derived from crossing wild-type pollen to emasculated *sin1-2/sin1-2* flowers. The seeds from individual flowers were germinated on agar. (E) Cleared whole mount of a rare normal seedling. (F–H) Cleared whole mounts of defective *sin1-2/+* seedlings derived from the same cross as in E. These have reduced hypocotyl, no root development (F, G), incomplete vasculature in the cotyledons, and highly reduced (G) or no cotyledon (H). (I) Postembryonic leaf buds on a normal seedling. (J) A seedling without postembryonic leaf buds. (K–Q) Scanning electron micrographs of 10-day postgermination seedlings obtained as above. The rare normal seedlings show two cotyledons, the primary and secondary leaf initials, a normal hypocotyl, and an elongated root (K). Abnormal seedlings (L–Q) show no postembryonic leaf initials (L, N–Q), a single cotyledon (L), tricotyly (O), a fused cotyledon shaped as a funnel (P), no root elongation (L, N, O, Q), and with no regular pattern (Q). (R–W) Confocal laser-scanning microscope analysis of the shoot apical axis of pregermination embryos stained with propidium iodide. Three 10-μm medial optical sections, 50 μm apart, are shown in R and S of an embryo from a wild-type flower and in U–W of a *sin1-2/+* embryo from a *sin1-2/sin1-2* flower, respectively. Note the presence of a dome of meristem cells in the wild type (arrowhead in S) but not in the maternally affected embryo. Size bars: A, 33 μm; B, 28 μm; C, 300 μm; D, 236 μm; E, 1400 μm; F, 730 μm; G and H, 500 μm; I and J, 44 μm; K, 2750 μm; L, 234 μm; M, 500 μm; N, 440 μm; O, 412 μm; P, 458 μm; and Q, 410 μm. Abbreviations used: *es*, embryo sac; *c*, cotyledon.

TABLE 1
Maternal Effects of *sin1*

Cross number	Maternal sporophyte	Egg	Sperm	Endosperm	Zygote	Number of seeds: Planted	Number of seeds: Germinated	Frequency of embryo development (%)
1a	+/+	+	*sin*	+/+/*sin*	+/*sin*	13	12	92
1b	+/+	+	*sin*	+/+/*sin*	+/*sin*	22	22	100
2	+/*sin*	+	*sin*	+/+/*sin*	+/*sin*	33	33	100
3	+/*sin*	*sin*	+	*sin*/*sin*/+	*sin*/+	22	22	100
4	*sin*/*sin*	*sin*	+	*sin*/*sin*/+	*sin*/+	293[a]	24[b]	8 (1% normal)
5	*sin*/*sin*	*sin*	*sin*	*sin*/*sin*/*sin*	*sin*/*sin*	297[a]	34[c]	11 (1% normal)
6	*sin*/+	*sin*	*sin*	*sin*/*sin*/*sin*	*sin*/*sin*	14	14	100

[a] Most seeds were collapsed and had brown seed coats. Approximately half of these had abnormal shapes. Seeds that fail to germinate contain embryos arrested at early developmental stages.

[b] Four seedlings were normal and grew to maturity (class A); 11 had two cotyledons, a root initial that failed to grow, and an expanded hypocotyl but produced no postembryonic leaves (class B); 1 had two cotyledons, an arrested root initial, a compressed hypocotyl, and no postembryonic leaves (class C); 1 had a single cotyledon, an arrested root initial, an expanded hypocotyl, and no postembryonic leaves (class D); 1 had a funnel-shaped cotyledon, a short hypocotyl, and arrested root (class E); 3 had no cotyledon, a short hypocotyl, and an arrested root initial; 1 had three cotyledons, a very short hypocotyl, and an arrested root initial (class F); 2 had no distinct pattern (class G).

[c] Three were class A, 16 were class B, 2 were class C, 1 each belonged to classes D and E, 8 belonged to class F, and 3 belonged to class G. The allele *sin1-2* was used in all crosses except in cross 1b where the stronger *sin1-1* allele was used.

normal (Figs. 1E, 1I, and 1K), but 42 showed various morphological abnormalities. The most pronounced effects were in the apical axis: seedlings with 0, 1, 3, or a funnel-shaped cotyledon(s) were frequently observed (Figs. 1G, 1H, 1L, and 1O–1Q). Many seedlings with 2 normal cotyledons never developed the primary leaf and usually never extended the root (Figs. 1F, 1J, 1N, and 1O). Confocal imaging of the apical axis of an embryo derived from a *sin1-2*/*sin1-2* sporophyte indicated that although it had two cotyledons, there was little or no meristem (Figs. 1U–1W). An extreme effect of the maternal mutation was seen in a few seedlings that were a chaotic mass of tissues without an obvious pattern (Fig. 1Q). The spectrum of morphological aberrations was identical whether the embryos were heterozygous or homozygous mutants so long as the sporophyte was *sin1-2*/*sin1-2* (see notes to Table 1). These results indicate that the wild-type *SIN1* gene in the maternal tissues either codes for or directs the synthesis of a diffusible product required for normal embryo development.

Epigenetic effects at the level of DNA modification have been shown to cause pleiotropic defects in meristem behavior and organ development (Ronemus *et al.*, 1996). Our results do not formally exclude a maternal epigenetic inheritance as the underlying mechanism of *sin1*'s maternal effects. We consider this latter explanation unlikely because the late-flowering phenotype of *sin1* shows no maternal effect: when a heterozygous embryo borne on a homozygous mutant flower grows to maturity, it shows no defect in flowering time. Conversely, a homozygous mutant embryo borne on a heterozygous mother plant is late flowering (Ray *et al.*, 1996).

Zygotically active genes such as *GNOM* (*EMB30*), *MONOPTEROS*, and *MICKEY* are important in multiple steps during embryogenesis (Mayer *et al.*, 1993; Berleth and Jürgens, 1993). Mutations in *gnom* and *monopteros* cause a range of defects in the cotyledons and the root initial, the same two embryonic tissues most frequently affected in the embryos growing within the *sin1* sporophyte. It will be interesting to determine whether a maternal *SIN1* gene interacts genetically with one or more zygotic genes for specifying apical–basal patterning in *Arabidopsis*.

A hallmark of maternal effect mutations in *Drosophila* genes important for signaling and transduction of positional information, such as those in *bicaudal, cappuccino, spire, decapentaplegic, dorsal,* and *notch,* is that the mutant phenotypes are highly pleiotropic and often show a range of variability (Nüsslein-Volhard, 1977; Shellenberger and Mohler, 1975, 1978; Mohler and Wieschaus, 1987; Manseau and Schüpbach, 1989). These properties were shown to result from chaotic disturbances in the positional information, in cell-specific responses to positional values, or due to distinct roles played by these gene products in different temporal and spatial contexts. Mutations in *sin1* are highly pleiotropic and show unexpected genetic interactions with *erecta* and *terminal flower1* mutations (Lang *et al.*, 1994; Ray *et al.*, 1996). The *ERECTA* gene codes for a putative serine–threonine receptor protein kinase (Torii *et al.*, 1996) and the putative ortholog of the *TERMINAL FLOWER1* gene in snap dragon codes for a predicted phosphatidyl ethanolamine-binding protein (Bradley *et al.*, 1996), indicating that both these genes are components of signal transduction pathways. The pleiotropism of *sin1* mutation, the existence of genetic interactions between *SIN1* and other genes important in signal transduction, and the maternal requirement of *SIN1* for embryonic pattern formation collectively suggest that *SIN1* may be important for cell to cell communication.

We propose that continued expression of the *SIN1* gene within diploid maternal cells, perhaps within those cells lining the embryo sac, is necessary throughout embryogenesis for coordinating pattern formation. Accordingly, the diffusible substance that acts on the embryo, whose production is regulated by maternal expression of the *SIN1* gene, is a morphogen. Although unlikely because the egg cytoplasm is small, we have not eliminated the possibility that the maternal *SIN1* gene encodes an mRNA that is deposited asymmetrically within the egg in a manner analogous to insect oogenesis.

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